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Research Report

Activated protein C analog with reduced anticoagulant activity improves functional recovery and reduces bleeding risk following controlled cortical impact

Corey T. Walker[a], Andrew H. Marky[a], Anthony L. Petraglia[b], Tracy Ali[c], Nienwen Chow[c], Berislav V. Zlokovic[a,*]

[a]Center for Neurodegenerative and Vascular Brain Disorders, University of Rochester Medical Center, Arthur Kornberg Medical Research Building, 601 Elmwood Ave, Box 670, Rochester, New York 14642, USA
[b]Department of Neurosurgery, University of Rochester Medical Center, Rochester, New York, USA
[c]ZZ Biotech, L.L.C., Rochester, NY, USA

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ABSTRACT

The anticoagulant activated protein C (APC) protects neurons and vascular cells from injury through its direct cytoprotective effects that are independent of its anticoagulant action. Wild-type recombinant murine APC (wt-APC) exerts significant neuroprotection in mice if administered early after traumatic brain injury (TBI). Here, we compared efficacy and safety of a *late* therapy for TBI with wt-APC and 3K3A-APC, an APC analog with ~80% reduced anticoagulant activity but normal cytoprotective activity, using a controlled cortical impact model of TBI. Mice received 0.8 mg/kg intraperitoneally of recombinant murine 3K3A-APC, wt-APC or saline at 6, 12, 24 and 48 h after injury. 3K3A-APC ($n=15$) relative to wt-APC ($n=15$) improved motor and sensorimotor recovery within the first three days post-trauma as demonstrated by rotarod ($p<0.05$) and beam balance test ($p<0.05$), respectively. Both, wt-APC and 3K3A-APC reduced the lesion volume seven days after injury by 36% ($n=8$; $p<0.01$) and 56% ($n=8$; $p<0.01$), respectively, compared to saline ($n=8$). Three days post-TBI, the hemoglobin levels in the injured brain were increased by ~3-fold after wt-APC treatment compared to saline indicating an increased risk for intracerebral bleeding. In contrast, comparable levels of brain hemoglobin in 3K3A-APC-treated and saline-treated mice suggested that 3K3A-APC treatment did not increase risk for bleeding after TBI. Thus, compared to wt-APC, 3K3A-APC is more efficacious and safer therapy for TBI with no risk for intracerebral hemorrhage.

* *Corresponding author*. Fax: +1 585 273 3133.
E-mail address: Berislav_Zlokovic@URMC.Rochester.edu (B.V. Zlokovic).

Abbreviations: TBI, traumatic brain injury; APC, activated protein C; PC, protein C; PAR-1, protease-activated receptor-1; EPCR, endothelial protein C receptor; BBB, blood brain barrier; CCI, controlled cortical impact; wt, wild-type; IP, intraperitoneal; PIB, pre-injury baseline; ANOVA, analysis of variance

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1. Introduction

Activated Protein C (APC) is an endogenous serine protease that participates in systemic anticoagulant activity (Griffin et al., 2002; Mosnier et al., 2007). This action is mediated by highly specific proteolytic degradation of factors Va and VIIIa, with contributions from various plasma cofactors (Mosnier et al., 2007). Independent of its anticoagulant pathway, APC exerts direct vasculoprotective and neuronal-protective activities that require proteolytic activation of protease-activated receptor-1 (PAR-1) and endothelial protein C receptor (EPCR) on endothelial cells (Cheng et al., 2003, 2006) and PAR-1 and PAR-3 on mouse cortical neurons (Guo et al., 2004, 2009a; Zhong et al., 2009) as well as PAR-1 and EPCR on rat hippocampal neurons (Gorbacheva et al., 2009, 2010). Cellular effects of APC include cytoprotective alterations in gene expression profiles, anti-apoptotic and anti-inflammatory activities and stabilization of endothelial barriers (Joyce et al., 2001; Riewald et al., 2002; Cheng et al., 2003; Domotor et al., 2003; Mosnier and Griffin, 2003; Feistritzer and Riewald, 2005; Finigan et al., 2005; Cheng et al., 2006). APC protects neurons and brain endothelial cells by inhibiting both the intrinsic and extrinsic apoptotic pathways (Cheng et al., 2003, 2006; Guo et al., 2004; Liu et al., 2004; Gorbacheva et al., 2010).

APC therapy has been shown to be neuroprotective in rodent models of transient brain ischemia (Shibata et al., 2001; Cheng et al., 2003, 2006) and embolic stroke (Zlokovic et al., 2005), neonatal hypoxic/ischemic brain injury (Yesisilirmak et al., 2008),compression-induced spinal cord injury (Taoka et al., 1998), ischemic spinal cord injury (Hirose et al., 2000; Yamauchi et al., 2006), multiple sclerosis (Han et al., 2008) and amyothrophic lateral sclerosis (Zhong et al., 2009). Following cerebral ischemia, APC can also promote angiogenesis and neurogenesis independently of its neuroprotective effects (Thiyagarajan et al., 2008). By using a controlled cortical impact (CCI) model of traumatic brain injury (TBI) in mice, we have recently shown that wild-type recombinant APC (wt-APC) given immediately after trauma improved functional outcome and reduced the lesion volume (Petraglia et al., 2010).

Although our recent study demonstrated potential of APC as a therapy for TBI (Petraglia et al., 2010), the anticoagulant properties of wt-APC may carry a significant risk for intracerebral bleeding, as shown in patients with severe sepsis (Bernard et al., 2001) and in rodent models of stroke when APC was administered at later time points after ischemia (Wang et al., 2009; Guo et al., 2009a,b). In contrast to wt-APC, late post-ischemic therapy with 3K3A-APC, an APC analog with greatly reduced anticoagulant activity (Gale et al., 2002), had superior beneficial effects and no risk for intracerebral bleeding after stroke in rodents (Guo et al., 2009a; Wang et al., 2009). 3K3A-APC contains 3 alanine substitutions for 3 protease domain residues (Lysine 191–193) which reduce factor Va binding and inactivation (Gale et al., 2002), but do not affect APC exosites recognizing PAR-1 and EPCR, resulting in normal cytoprotection (Mosnier et al., 2004). In the present study, we compared efficacy and safety of a late therapy for TBI with wt-APC and 3K3A-APC.

2. Results

2.1. Motor and sensorimotor recovery following CCI

A series of tests were performed on consecutive days following CCI to evaluate functional motor recovery in all groups. In the rotarod assessment of motor activity (Hamm et al., 1994), both 3K3A-APC-treated ($n=15$ mice) and wt-APC-treated ($n=15$ mice) groups demonstrated significant improvements relative to vehicle-treated mice ($n=10$ mice) until post-injury day 7 when performances leveled off ($p<0.05$, ANOVA; Fig. 1A). This finding was consistent with a previous report using an early post-TBI treatment with wt-APC (Petraglia et al., 2010). On day 1 post-TBI, mice treated with 3K3A-APC had an average pre-injury baseline latency of 69% compared to 52% in mice treated with wt-APC, and 27% in controls, suggesting that 3K3A-APC relative to wt-APC enhances motor performance ($p<0.05$, Tukey post

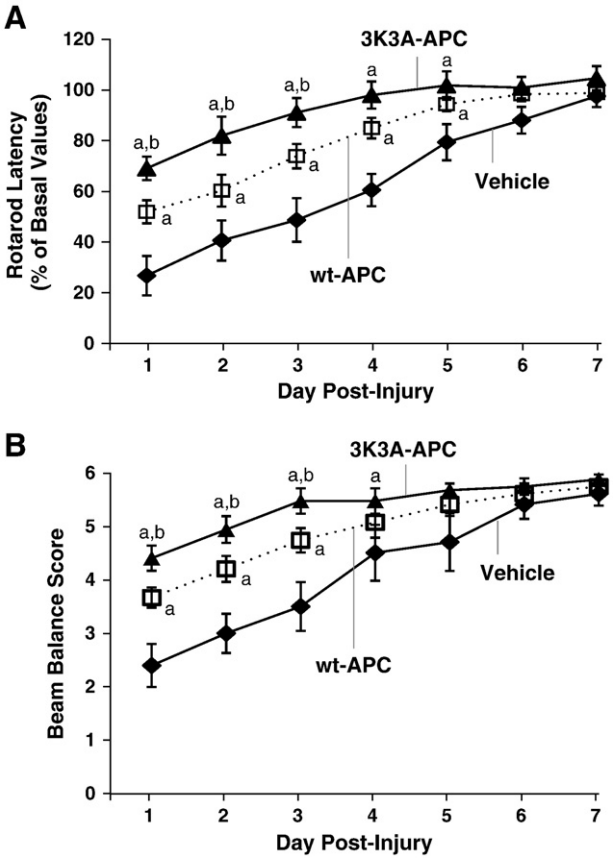

Fig. 1 – Effects of 3K3A-APC and wt-APC therapies on motor neurological and complex sensorimotor outcomes determined by rotarod neuromotor (A) and beam balance (B) tests, respectively. 3K3A-APC or wt-APC were administered at 0.8 mg/kg I.P. 6 h after injury and subsequently at 12, 24 and 48 h after injury. Control mice were treated with saline (vehicle). Data are means ± S.E.M., $n=15$ for APC-treated mice/groups, and $n=10$ for saline-treated mice; [a]$p<0.05$ for 3K3A-APC or wt-APC compared to saline; [b]$p<0.05$ for 3K3A-APC vs. wt-APC, Tukey post hoc test.